

Chapter 14

Digits, segments, somites: the superposition of periodic and sequential structures

A type of structure which is frequently encountered in higher organisms consists of a sequence of similar but not identical substructures (Fig.14.1). For example, the segments of insects, separated by segment borders, are arranged in a repetitive manner. In the vertebrate limb, areas of presumptive digits and of programmed cell death alternate. However, each digit or each segment is different from the other, and is a member of a sequence of these substructures. Other examples are the bones of the backbone of vertebrates which originate from the sequence of somites.

The assumption of a graded distribution of a morphogen and the interpretation of this positional information has enabled us to explain many experiments concerning the determination of the insect segments (chapter 8), the segments of insect legs (see Figs. 9.2 and 9.4) and of vertebrate limbs (Figs. 10.1 and 10.7). In these models, the periodic aspect of these structures has been neglected. In

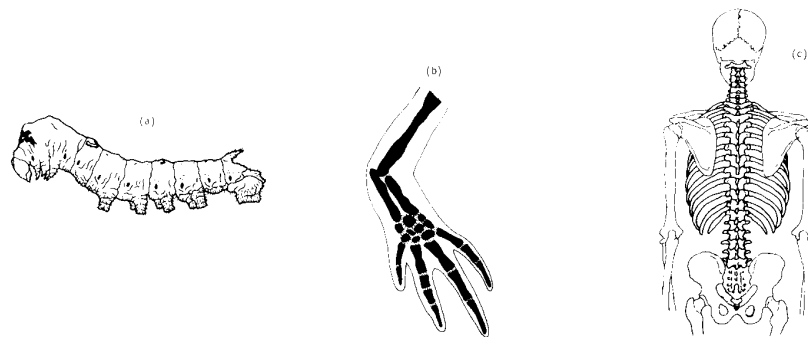


Figure 14.1: Superposition of sequential and periodic structures - a basic pattern in higher organisms. Sequences of similar but not identical subunits form more complex structures. Biological examples: (a) the segments of an insect (a silkworm, drawn after Tazima, 1964), (b) the digits of an amphibian limb and (d) the vertebrae of a human being.

this chapter we will see how the periodic alternation between two or three alternative states enables in a gate-like manner the transition from one state in a sequence to the next in a very reliable way.

To see which type of mechanism can account for the generation of such dual structures we will again refer to the insect system, especially to *Drosophila*, since the most detailed experimental observations are available there. It will turn out that the mechanism derived from the insect system is able to explain observations in the somite system, indicating that the generation of sequential and periodic structures in precise register is a very basic mechanism in development.

14.1 The formation of the periodic pattern is the primary event

In the thoracic segments of insects, almost simultaneously with the clonal separation into segments at the blastoderm stage, a separation into anterior (A) and posterior (P) compartments takes place (Garcia-Bellido et al., 1973, 1976; Steiner, 1976; Wieschaus and Gehring, 1976). Presumably they are arranged like zebra stripes and each segment contains one pair of A-P-stripes. Both patterns are in precise register. For instance, the border between Mesothorax to Metathorax is also always a P-A border. Both patterns must arise in a coupled process. The question is then, which process is the primary event. Either initially the sequence of segmental specifications 1,2,3... is formed and each region is later subdivided into an A and a P region (1A, 1P, 2A...) or the primary event is an A-P-A... pattern and each pair of stripes obtains in a secondary process a particular segmental specification (Fig.14.2). An answer to this very important question can be obtained from mutants in the control gene region responsible for the metathoracic specification, the Bithorax gene complex (Lewis, 1963, 1964, 1978; Sander, 1981). If, for instance, the locus *Cbx* or *bx* is mutated, a particular segmental specification extends into an adjacent segment without changing the A-P-A pattern (Fig.14.3), indicating that the A-P-A pattern is independent of the segmental specification and that the formation of the A-P-A pattern is the primary event. It is rather the coupling of the segmental specification to this A-P-A pattern which is abolished in the mutation of the Bithorax gene complex (BX-C). In this chapter, we will see how this coupling is achieved during normal development and how particular transformations come about after a failure of particular elements in this switching system. That the periodic subdivision is the primary event appears also reasonable from an evolutionary point of view. The insects evolved during evolution from lower Arthropodes and Annelides, creatures with many similar segments, indicating that the repetition of almost identical subunits was an early evolutionary achievement while diversification of the segments is a latter event.

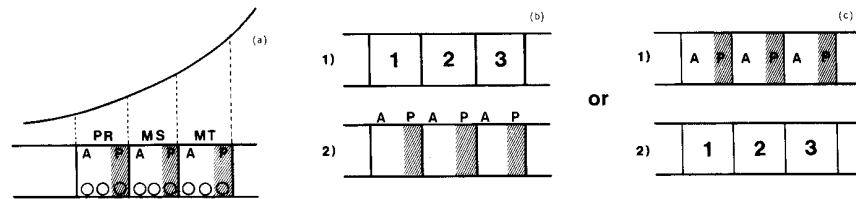


Figure 14.2: Sequential and periodic pattern - what is the primary process? (a) In the thoracic segments of *Drosophila* the periodic pattern of anterior (A) and posterior (P) compartments is in register with the segmental specification (1,2,3...). The transition from the Meso- to Metathorax (MS-MT) coincides precisely with a P-A transition. This indicates that either the sequential pattern is the primary event and the A-P stripes are formed as a subpattern (b) or the formation of the periodic A-P structure is the primary event, and under its influence, each A-P pair gets a particular specification (c).

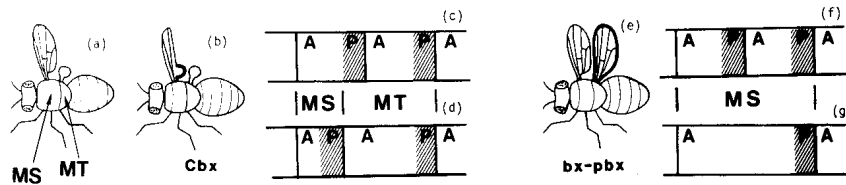


Figure 14.3: Evidence that the periodic subdivision is the primary event. (a) Schematic drawing of a wild type of *Drosophila*. (b,c) In the *Cbx* mutant the region of metathoracic specificity (MT) is enlarged at expense of the mesothoracic (MS) region. Neither the A-P pattern nor the segmentation is affected (c). If the A-P subdivision were the secondary event, a subdivision of the smaller MS region into A and P and the subdivision of the larger MT region in only two compartments would be expected, as drawn in (d). (e,f) Similarly, in a *bx pbx* double mutation two pairs of AP stripes become MS specificity (f). If the A-P-pattern were a secondary subdivision, only one AP pair (perhaps larger) with MS specificity would be expected (g).

14.2 “Gating” of the transition from one control gene to the next: the pendulum - escapement - model

Assuming that the formation of the periodic structure is the primary event, a mechanism accounting for the precise superposition of both periodic and sequential structures must have the following features (Meinhardt, 1981): (i) It is able to create a stable periodic structure, possibly stripes, (ii) the alternation of stripes controls the segmental specification and (iii) the number of repetitive elements and therefore the width of the stripes is under the control of a morphogen gradient.

The mechanism envisaged can be illustrated by an analogy. Imagine a grandfather clock. The weights are at a certain level (corresponding to the local morphogen concentration). They bring a pendulum into motion which alternates between two extreme positions. The escapement mechanism allows the hand of the clock to advance one unit after each change from one extreme to the other. The periodic movement of the pendulum is the primary event and the movement of the hand of the clock is under its control. As the clock runs down, the number of left-right alternations of the pendulum and hence the final position of

the pointer is a measure for the original level of the weights (level of morphogen concentration). In terms of the mechanism for the interpretation of positional information, we will assume that, under the influence of the morphogen, the cell alternates between two states, to be called A (anterior) and P (posterior) and that the total number of alternations corresponds to the level of the local morphogen gradient. Under the influence of this alternation, for instance at each P-A transition, the cell switches stepwise from one specification i to the next, $i+1$ ($i=0,1,2\dots n$). The stepwise advancement from one state to the other under the influence of the alternation between P and A may be compared with a ship in a channel with locks. A lock can be in two states. Either the upper gate is open and the lower gate is closed or other way round. In one state, the ship can enter into the lock but it can pass only after a switch into the other state. One state is characterized by the preparation, but blocking, of the transition. The other state enables the transition but no entrance into the next preparative phase is possible. This enabling and blocking of transitions by the alternation between two states we will call “gating”. Only a full cycle of alternation allows an progression of one and only one step. In this way, the graded morphogen concentration becomes converted into the alternating A-P-sequence and into the sequence of structures 0,1 ... n. Since both patterns are formed in this coupled way, they are necessarily in register. A particular state of a cell can be characterized by its A-P state and its specification, for instance, 1A, 1P, 2A and so on. Cells exposed to a lower morphogen concentration obtain their final determination earlier, after a few alternations while distal determinations require more time. This is in agreement with the stepwise and unidirectional “promotion” of the cells under the influence of the morphogen which was concluded from the insect experiments (see Fig.8.7).

The following elements are required for the realization of the model:

- (1) The cells can be in one of two states (A or P). The transition from at least one of these states to the other, for instance P to A, requires a threshold morphogen concentration. The alternative transition (A to P) can be an autonomous process like the swinging back of a pendulum.
- (2) The advancement from one specification, that means from one structure-controlling gene activity, to the next (i to $i+1$), proceeds under the influence of such a transition, e.g. P-A.

14.3 The oscillation between A and P and the generation of stable A-P-stripes

The fact that an anterior fragment of a leg disk can regenerate posterior compartmental specifications indicates that the periodic arrangement of compartments is a dynamically stable system (see Fig. 9.2 and 9.3). We have seen (chapter 12, Fig. 12.2) how stripes can be formed and stabilized. The basic idea was that two states, to be called A and P, exclude each other locally but at long range help each other and depend on this help. This necessitates that both structures

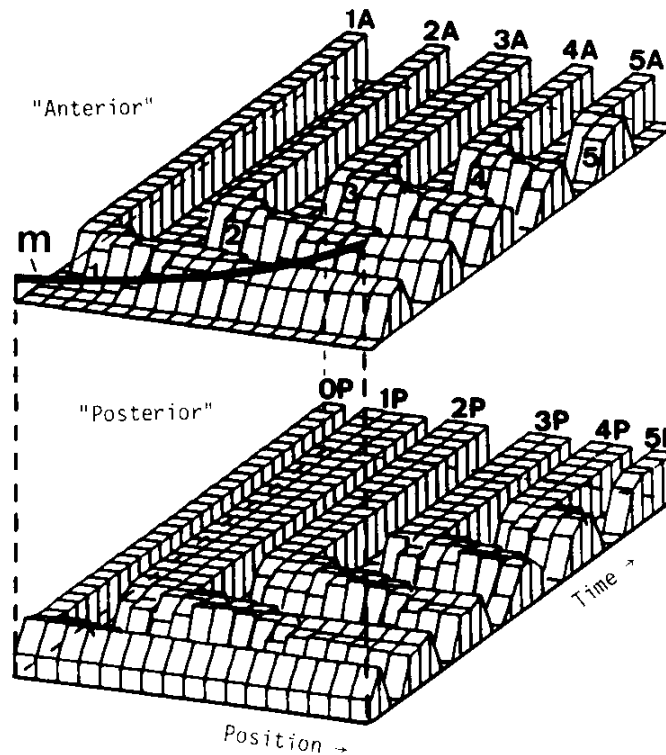


Figure 14.4: The P-A-P oscillation under the driving force of a morphogen gradient. The simulation should demonstrate that the number of P-A transitions a cell has made in its developmental history is the same as the number of the A-P stripe in space in the finally stable pattern. Initially, all cells are assumed to be in the P-state. Those cells exposed to a certain morphogen concentration (m) switch to A; the first P-A border is formed. Those A cells distant to this border are not stabilized and switch back to P, forming a second (A-P) border. With each further oscillation, a new stable A-P stripe is formed. If for each further P-A transition a slightly increased morphogen concentration is required, the width of the A-P stripes is determined by the gradient (Fig. 14.10). The final result is a stable regular A-P-A pattern. Nevertheless, this A-P pattern has self-regulating features: An isolated patch of A-cells will reestablish an A-P pattern (Fig. 12.6)

are formed in close proximity to one another. A stripe-like pattern is especially favored since, in this case, the long common boundary regions enable an effective mutual stabilization.

While A and P cells stabilize each other in the region of a common boundary, it is a property of such an interaction that a group of cells consisting of one type only (A or P) can oscillate back and forth between the two possible states. If, for instance, all cells are in state A, the state P gets an enormous help while the state A is not supported. After a certain time, the cells switch from A to P. Later, the cells switch back to A for the same reason.

This spatially homogeneous oscillating system would be converted into a pattern which is stable in time if, at any location, an A-P border has been formed. Imagine a linear array of cells, which are under control of a graded morphogen

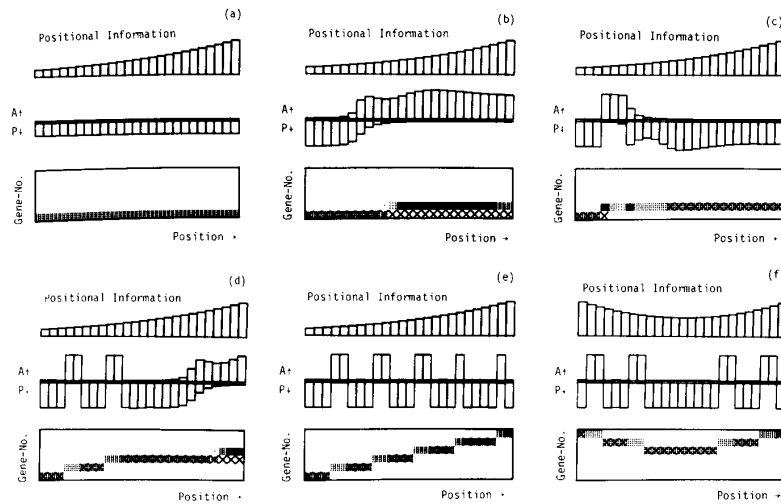


Figure 14.5: Stages in the interpretation of positional information according to the pendulum-escapement model. The antero-posterior gradient (top of each subpicture) provides positional information. (a) Originally, all cells are in the P state and gene 0 (coding e.g. for extraembryonal development) is active. In the P state, a substance is produced which activates the next following control gene (x) but this transition is blocked. (b) All cells exposed to a threshold concentration switch to the A state and therewith from gene 0 to gene 1 (since in the A-state the transition is not blocked). A and P cells need each other in the neighborhood for mutual stabilization. Those A cells without P neighbors switch back to the P-state (c). For the next P-A switch, an increased morphogen concentration is required. This process will continue (d) until the total field is subdivided in the periodic pattern of anterior and posterior specifications and into the sequential pattern of gene activity. (f) Simulation of a “bicaudal” embryo (see Fig. 8.3). A computer program for these simulations is provided in chapter 17.

concentration. All cells are in the P state and a certain morphogen concentration is required to induce the first P-A transition. Cells exposed at least to the threshold concentration switch from P to A and form in this way the first P-A border. Cells close to this border stabilize each other while the A cells distant to this border switch back to P, forming in this way a second A-P border. Again, cells distant to this new boundary will switch back to A, and so on. After each full cycle, one pair of A-P stripes is added. As the process progresses, the region of the stable spatially alternating A-P pattern enlarges at the expense of the spatially homogeneous cells which oscillate between A and P in time. The borderline between the stable and oscillating cells move over the field in a wave-like manner. This mechanism will continue until the total area is subdivided into a stable spatial periodic A-P pattern. A biological system in which repetitive structures are formed visibly in a wave-like manner is the genesis of somites of vertebrates. It will be discussed below in more detail.

14.4 Switching to new a control gene under the influence of posterior-anterior (P-A) transition

After a stable state is reached in a particular cell, the number of oscillations a cell has made in its history is the same as to the number of the stripe it belongs in space (Fig.14.4). The unequivocal correlation between the number of oscillations a cell has made and its position in the field enables a reliable activation of a particular control gene in a particular stripe, for instance, by the following mechanism. In the P state, the switching to the next control gene prepared but the transition is blocked ("posterior block"). In the A-state, the transition is no longer blocked and the next following control gene becomes activated. However, no attempt is made in A to activate the subsequent control gene. As explained above with the ship and lock analogy, this has the consequence that a transition from one control gene to the next is possible only during a P-A transition and the control gene which finally becomes activated, depends on the total number of P-A transitions (Fig.14.5). Let us assume that all cells are originally in the gene-0, P-state (0P). Only those cells which are exposed to a sufficient morphogen concentration will switch to A. Since it is a P-A transition, the cells switch from specification 0 (corresponding, for instance, to extraembryonal development in insects or to the anterior necrotic zone in digit formation) to the state 1. The 0P and 1A cells stabilize each other, while cells further distant switch from 1A to 1P as described above. If the threshold remain unchanged, a periodic structure would be formed as described above since the next 1P-2A transition would take place in a region of even higher morphogen concentration. It is conceivable, however, that a certain incremental increase in the next P-A threshold results from the previous 0-1 transition. If this were so, a definite increment in the morphogen concentration would be required and the steepness of the gradient would determine the width of a pair of stripes.

14.5 Expected mutations and the phenotypes of the Bithorax complex of *Drosophila*

The best investigated complex of genes controlling a particular segmental specification is the Bithorax gene complex of *Drosophila* (Fig.14.6; Lewis, 1963, 1964, 1978; Garcia-Bellido, 1977). At the first glance, the phenotypes of the mutants appear quite puzzling. For instance, the anterior or the posterior haltere may become transformed into the corresponding part of the wing, flies with two wings can appear, the first abdominal segment may be transformed into mesothoracic structures and bear a fourth pair of legs, and so on. Why in most cases is only one half of a segment transformed? Why do these transformations respect compartment boundaries? Why do they cause, as a rule, a transformation into a structure of a neighboring segment? The analysis of the phenotypes led me to the pendulum model as described above and after finding it, the mutations of the Bithorax complex (BC-X) appear to be the consequence of an underlying prin-

ciple and not just an accumulation of genetic modification collected during the evolutionary history. It should be shown that the mutations are explicable under the assumption that the BX-C is the control gene for the Metathorax (MT) and that its activation is gated by A-P-A changes. To see which type of mutants we expect on the basis of the model, the stepwise transition from one control gene to the next under the P-A alternation should be compared with the passage through a series of rooms. All the rooms are separated by doors. Each evening (P-state), one can proceed to the next door and ring the door bell. The next morning (A-state), the corresponding door will be opened. One can enter into the room and the door will be closed behind. However, one cannot proceed to the next door. This is possible only the following evening. The numbers of rooms someone has passed would correspond to the number of day-and-night cycles. The following types of “mutations” are expected:

- (1) “Broken door”. One can enter into the next room too early, already at the night before. If each room has two doors, one broken door is sufficient for an entry too early - the mutation is dominant.
- (2) “Broken door bell”: The door will not be opened correctly with the P-A transition. One remains in the last room. If two door bells are present, one is sufficient to ring the bell - the mutant is recessive.
- (3) “Last door left open”: One enters into the next room but the door cannot be closed behind. The new room has partially the character of the previous room.

The arrangement of the alleles on the chromosome as well as - according to the model - their normal function and transcription are shown in Fig.14.6. Hayes et al. (1979) have proposed that the BC-X genes are transcribed from an operator region in the Ubx^+ region and that the direction of transcription depends on the compartmental specification of the particular cell. I will follow this proposal. Transcription is to the left (proximally) in the anterior compartment, thus enabling the transcription of Cbx^+ and bx^+ while in the posterior compartment, it is to the right, causing the transcription of bx^+ and pbx^+ .

To correlate the particular loci of the BX-C with particular functions in the model we have to compare the expected and observed mutations. In the PMS (posterior Mesothorax) we expect that an attempt to activate the control gene for the MT, the BX-C, is made but that this activation is blocked, for instance by a transcriptional block of the BX-C. A failure of this block (“broken door”) will be a dominant mutation and lead to MT structures in the PMS segment. That is the phenotype of the Cbx mutation (Fig.14.7). The Cbx^+ region is assumed to prevent the transcription of the bx region in a P-state. After a switch to the A-state, this posterior transcriptional block (PB) is released and bx can be transcribed. With this P-A transition, a transition from MS to MT specification should occur: We will assume therefore that wildtype function of bx is to suppress the MS pathway. If bx is mutated (bx^-), a MS-repressor cannot be produced in the AMT segment.

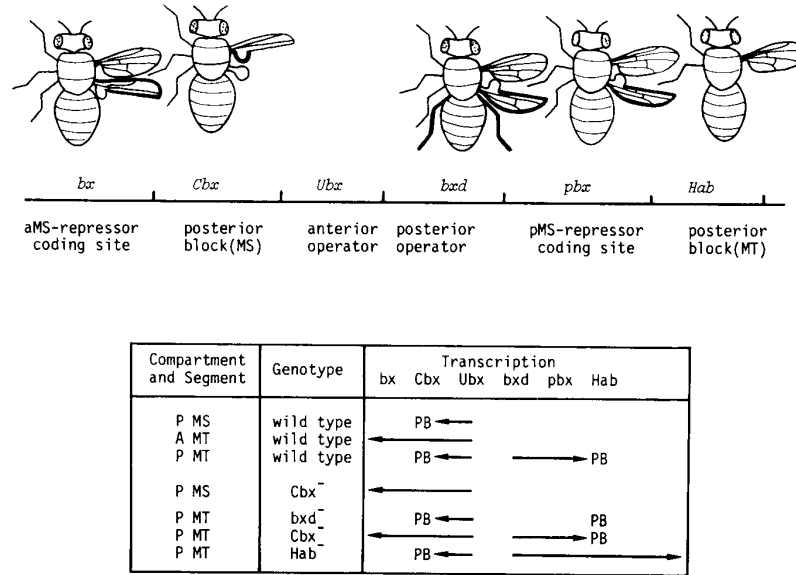


Figure 14.6: The phenotypes of the mutants of the Bithorax gene complex (BX-C, Lewis, 1963, 1964, 1978) of *Drosophila*, their arrangement on the third chromosome and the normal function according to the model proposed. Abnormal structures are drawn with heavy lines. The arrows in the scheme below indicate the proposed transcription of the BX-C as function of the segmental and compartmental specifications. In the posterior state, the termination of transcription results from a posterior transcriptional block (PB). Mutation of the PB-sites (Cbx and Hab) can change the extent of transcription.

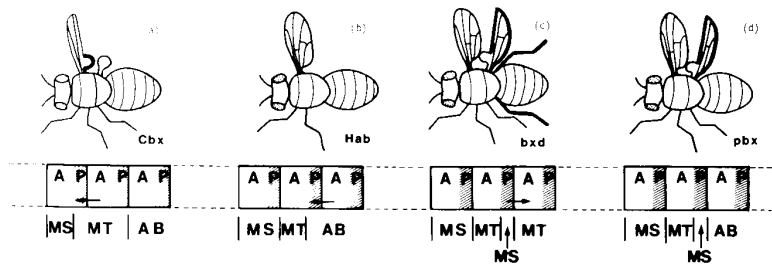


Figure 14.7: Types of transformation expected from the model and observed in mutations of the Bithorax gene complex. (a,b) The posterior block does not work; the transition into the next posterior segment specification occurs already at the A-P transition. The segmental specificity is extended into the more anteriorly located segment. Corresponding phenotypes are *Cbx* (a) and *Hab* (b). (c) The activation of the next following control gene does not work correctly, the same segmental specification is repeated in the posteriorly located segment. This is the phenotype of *bxd*. (Important is in this context the formation of a fourth pair of leg. The PMT-PMS transformation results from a particular arrangement of the loci on the chromosome.) (d) The correct transformation control gene is activated in the correct region but the previously active gene is not suppressed. An example is *pbx*. Note that this is not an extension of a particular specification into a neighboring segment.

The AMT segment receives AMS character despite that the correct control gene is activated ("last door left open"). In contrast, in a *Cbx* mutation, the *bx* gene is already transcribed in the PMS segment, the MS repressor becomes produced and a PMS-PMT transformation occurs. With these assignments, the phenotypes of *Cbx*⁻ and *bx*⁻ are explained.

After an A-P transition in the MT segment, the *Cbx-bx* transcription is blocked again at the *Cbx* region. A second coding region for the MS repressor, transcribed in the PMT segment, is required. This is assumed to be the *pbx* region. A *pbx* mutation therefore leads to an PMT-PMS transformation. The transcription of the *pbx* gene is assumed to start in the *bx**d* region. A mutation in the *bx**d* region leads therefore to a loss of function of a *pbx*⁺ gene (Fig.14.6, 14.7). In the PMT region, the activation of control genes responsible for abdominal (AB) structures must be prepared. In PMT, the transcription is assumed to proceed from *bx**d* via *pbx* towards AB-genes. However, in the PMT region the transcription of the AB genes is blocked by a second posterior block at the *Hab* region. In *Hab*⁻ flies, this block fails and abdominal genes are already activated in the PMT leading to a loss of the haltere and of the third pair of legs. Since *Hab*⁻ is of the "broken door type", it is dominant. In contrast, *bx**d* is required to activate the AB genes; *bx**d*⁻ is therefore of the recessive "broken door bell" type and leads to an repetition of thoracic structures in the first abdominal segment. In *pbx*⁻, the correct control gene is activated but the product, the MS repressor, does not work; *pbx*⁻ is therefore of the type "last door left open". The gating mechanism is especially obvious in this part of the BX-C since the control genes are arranged on the chromosome in the same order as the corresponding structures in the real organism.

The model describes also the behavior of double mutants. For instance, a fly carrying a *Cbx* and a *pbx* mutation shows a pure *Cbx* phenotype. It does not matter whether *pbx* is mutated or not. The defects are not additive. According to the model, due to the *Cbx* mutation, the MS repressor coding region at *bx* is also transcribed in the PMT region and the MS pathway is suppressed independent of *pbx*. This double mutation suggests that the *bx*⁺ region is not a specific "selector gene" (Garcia-Bellido, 1975) for the AMT pathway but that it codes for a general MS repressor. The influence of the *bx* mutation is usually restricted to the AMT since, according to the model, *bx* is only transcribed there. The *Cbx* mutation is known to be an inverted insertion of the *pbx* region. I presume however that the mutant phenotype results not from this copy but from a destruction of a transcriptional block by this insertion.

The phenotype of double mutations frequently depends on whether the two mutations are located on the same chromosome (*cis*) or on different chromosomes (*trans*) and these differences are also correctly described by the model. For instance, if a *bx* and a *Cbx* or a *Ubx* and a *Cbx* mutations are located on the same chromosome (*cis*), the phenotypes are almost wildtype although *Cbx* alone would be dominant. According to the model, if the transcription cannot start (*Ubx*⁻) or the product is bad (*bx*⁻) it does not matter whether the posterior block at *Cbx* works or not. In contrast, if *bx*⁻ and *Cbx*⁻ or *Ubx*⁻ and *Cbx*⁻ are located

on different chromosomes (trans), on one chromosome the transcription starts correctly (Ubx^+), it is not blocked in the PMS segment (Cbx^-) and the product (bx^+) is good. The normal Cbx^- phenotype results. Or, if bxd^- and pbx^- are in cis, the other chromosome can take over all functions and the phenotype is wildtype. In trans, the transcription cannot start on one chromosome (bxd^-) while, on the other, the product is bad (pbx^-) and a pbx^- phenotype results, in agreement with the experimental findings (Lewis, 1963, 1964).

If, due to a chromosomal deletion, the BX-C is completely absent, the MT and all abdominal segments are of MS character (Lewis, 1978). In terms of the model, if the chain of sequential activation of control genes is interrupted at one step, the following genes in the sequence can be no longer activated. This does not mean that the BX-C genes are active in the abdominal segments. The activation of the BX-C can be a transient but necessary step in the activation of genes controlling abdominal structures.

The model allows to understand other experimental observations it was not intended to explain. A striking asymmetry occurs in the regeneration of compartmental specificities. An anterior leg compartment can regenerate the posterior compartment but the reverse regeneration occurs much less frequently if at all (Schubiger and Schubiger, 1978). A similar asymmetry has been reported by Kauffman and Ling (1981) for the wing. In terms of the model, an A-P transition will occur whenever the stabilizing influence of the P region on the A state becomes too low. In contrast, a P-A transition would require the driving force of the morphogen and is therefore less likely to occur in an isolated disk fragment.

The assignment of very specific functions to the BX-C is necessarily speculative. Modifications are expected from a more complete understanding about how the segmentation proper is controlled (see below). Corrections may become necessary with the determination of the DNA sequence of the gene complex. It is hoped, however, that the general principle, the sequential activation of control genes by the alternation between two states, holds and facilitates an understanding of the information obtained from the sequencing of the DNA.

14.6 Sequential addition of new units at a zone of marginal growth

In some insects, only a fraction of the segments is formed directly. Then, in a second step, pattern formation is completed by adding new segments at a zone of marginal growth. The number of elements formed during the first or second phase is very different in different species (Krause, 1939). For instance, in *Euscelis* (Sander, 1976), only very few abdominal segments are added by growth, and the pattern formation can be essentially described as under the control of a morphogen gradient (see Fig.8.2 and 8.7). In contrast, in crickets (Fig.14.8), most of the segments are formed by marginal growth. Thus, some insects are organized by morphogen gradients during an essential non-growing period while others during a period of substantial growth. Smooth transitions exist between

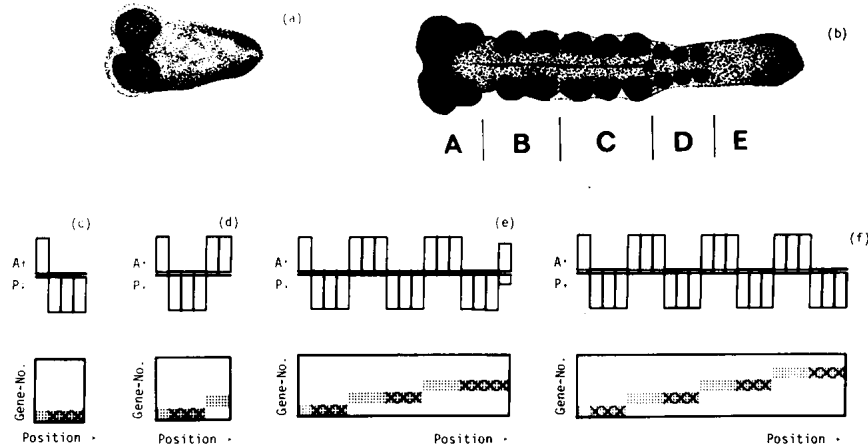


Figure 14.8: Formation of a sequential and periodic structure by marginal outgrowth. (a,b) Biological example: stained germ band of a cricket. At an early stage (a), only the head lobe (A) is separated. Later (b), head segments producing mouth parts (B), the three thoracic segments with leg buds (c), and three abdominal segments (D) are formed. More abdominal segments will be formed in a sprouting-like process from the not yet segmented area E (after a photograph of P. Bader, see Sander, 1981). (c-f) Model: during outgrowth, whenever a particular state (A or P) surpasses a certain size, a switch into the alternative state (P or A) occur. Each P-A transition can cause a transition to a following control gene. If marginal growth is involved, no positional information is required. A computer program for this simulation is provided in chapter 17

the two modes. This suggests that minor changes in the assumed mechanism should allow a pattern formation according to the one or the other regime. That is the case in the proposed gating mechanism. Let us assume a growing marginal A-area. Whenever some A cells become too remote to stabilizing P cells, they switch from A to P (and vice versa). The switching can be used in the same way as described above to gate a transition from one control gene to the next. In Fig.14.8, a simulation of a growing system is provided together with a biological example. Growing systems do not depend upon a gradient system which provides positional information since the order of the segmental specifications is determined by the growth.

Another pattern which is formed during marginal growth is the proximo-distal pattern of a vertebrate limb (see Fig.11.7, 11.8). Summerbell et al. (1973) have proposed a progress-zone model according to which the dividing cells at the growing tip count the number of cell divisions and acquiring with each division a more posterior positional value. Cells leaving this zone of cell division maintain their once obtained positional value. On principle, such pattern formation can be also described by the gating model. The primary subdivision would not be the subdivision in the sequential structure (Humerus, Ulna etc.) but in a periodic structure (for instance, bone, joint, bone... or proximal, distal, proximal... part of a bone). Coupled to each (or each second) switch from one state to the other, a new specification in the sequential pattern is determined. However, the pattern regulation after removal of internal structures in the amphibian leg indicates that

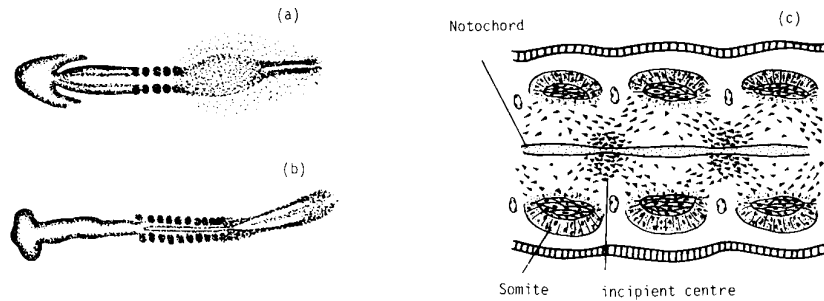


Figure 14.9: Formation of somites and vertebrae. Somites are formed by clustering of cells. It starts at the anterior side, behind the head lobe and proceeds in posterior direction. (a) A chicken embryo at about 25 h of incubation. 5 somites are visible. (b) Ten hours later, 12 somites are present. (c) The anterior and the posterior part of each somite appear to be different. For the formation of vertebrae, cells from the anterior part migrate in anterior direction while cells from the posterior part migrate in posterior direction. Thus, cells from two different somites form together one vertebra (redrawn after Patten, 1958).

positional information is involved in the leg system (see Fig.11.9b,e).

14.7 The formation of somites

A very important step in the antero-posterior organization of vertebrates is the formation of somites which give rise to the axial skeleton and musculature during further development. The paired somites are derived from two stripes of mesodermal tissue by a sequential separation into groups of cells. The separation progresses from anterior towards posterior. The periodic nature of somites is obvious (Fig.14.9). Similar to the thoracic segments of insects, each somite seems to be subdivided into (at least) an anterior and a posterior portion since cells originating from the posterior half of one somite together with cells from the anterior half of the next somite form one vertebrae (Fig.14.9c). Presumably the somites are also different from each other since the vertebrae arising in this process are different from each other. Particular vertebrae form ribs while other do not.

Many experimental observations have provided insights into how the formation of somites is controlled. In amphibians, the first ca. 20 somites are formed by a grouping of existent cells. Later, a graded transition to a more progress-zone like addition of new somites in a region of cell proliferation in the tail bud occurs (Cooke, 1975a). The mode of segmentation is therefore similar to that in insects discussed above. Although the first somites appear long before the last somites, the size of the somites is regulated in such a way that the number of somites is almost constant and independent of the size of the embryo at the blastula stage. For amphibians, Cooke (1981b) has shown that only the first anterior somites (about 20) are size-regulated while the more posterior somites are always smaller but independent of the size of the embryo. The actual determination of the somites occurs earlier than their morphological appearance. Both

processes can be experimentally distinguished by short heat shocks. In *Xenopus*, such a heat shock leads to defects of those somites which are formed at least 10 h later. Meanwhile, five normal somites appear. This indicates that the (heat-sensitive) determination of somites precedes their appearance by approximately 10 hours. An important question is whether the determination or the morphological separation of a particular somite require an inductive trigger from the previously formed anterior somite(s). Such a sequential trigger would explain the wave-like spreading of somite formation. This question has been answered by removing fragments from the posterior part of an amphibian embryo at the neurula stage. In this stage, the somites are neither determined nor visible. The surprising result is that in such fragments, the formation of somites takes place in the same sequence and at the same time as in the unoperated embryos. This indicates that the formation of one somite does not require its previously formed anterior neighbor. The actual somite formation occurs after a count-down-like process (Deuchar and Burgess, 1967; Pearson and Elsdale, 1979). However, in the heat shock experiments mentioned above, the number of malformed somites is much higher than expected from the shortness of the heat shock. Taking both observations together, the time at which the separation of the somites becomes determined and morphologically manifest seems to be cell-internally encoded. In the generation of fine structure, however, a neighboring interaction seems to play an essential role in such a way that an irregular shape or a fusion of some somites has an influence on the successively formed somites. It requires the formation of several somites until the once evoked disturbance is smoothed out.

In summary, a model which should account for somitogenesis must have the following features: (i) A periodic structure is formed in an anterior to posterior order. (ii) The individual somites formed in this process are different from each other. (iii) Each somite is subdivided (at least) in an anterior and posterior part. (iv) The size of the first anterior somites is controlled in relation to the total size of the embryo, the more posterior somites are of constant size. (v) The time at which the separation of somites occur is cell-internally determined. (vi) Neighboring interactions play a role in the generation of the fine structure.

With minor modifications, the model for compartmentalization and specification in insects discussed above has all these properties. Cooke and Zeeman (1976) proposed a model for somite formation in which an oscillator gates a wavefront. In the model I propose, the oscillator (alternating between A and P), the wave front (separating stable and oscillating cells) as well as the spatial periodic pattern (of A and P) results from one and the same mechanism. In addition, the model I propose accounts for a different determination of the individual somites. As in insects, the oscillation seems to be under the control of a gradient with increasing concentration towards the posterior end of the organism. In vertebrates, this gradient appears to be relatively stable since the local values remain unchanged after isolation of fragments. The absolute level of this gradient would determine after which time or, more precisely, after how many A-P-A transition a stable boundary can be formed (Fig.14.10). The formation of such stable boundary is assumed to correspond to the somite determination as discussed above.

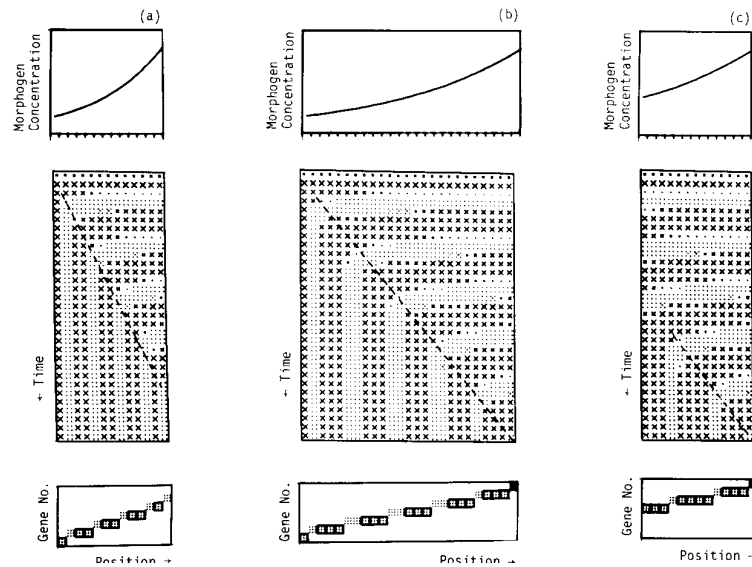


Figure 14.10: The gating mechanism as a model for somite determination. The same mechanism as in Figs. 14.4 and 14.5 is assumed. (a,b) A gradient with the same concentration range leads to a sequential and periodic pattern which is, within some limits, independent of the total size of the field. The upper subpictures show the assumed positional information, the central figure shows the oscillation between “A” (:::) and “P” (xx) and the formation of a stable periodic A-P-A pattern. The broken lines mark the transition from the oscillating into the stable state and indicate, therefore, the moment of somite determination as function of time and position. The lower subpicture shows the final pattern of gene activities. A square indicates that the corresponding cell is in the P-state. The time required to form a somite does not depend on the size of a somite but on the A-P oscillation frequency. (c) A posterior fragment; no area of low positional information is present. The first A-P boundary can be formed only after a certain number of synchronous oscillation and switches to higher “genes”. Separation into somites occurs therefore later, at the time corresponding to the local positional information (for computer simulations see chapter 17).

The steepness of this gradient would determine the size of the individual somites. (How a size-regulated gradient can be formed has been discussed above, Fig.7.1.) If an increase of the threshold for the next P-A transition occurs only with the determination of the first (ca. 20) somites, only these first somites will adapt to the steepness of the gradient. The activation of different control genes may be required only for the more anterior somites since they have to form structures with more individuality like ribs while the more posterior somites which forms the tail may be more or less identical. If the threshold is not increased, the A-P-A pattern is formed at the smallest possible distance. The simulations in Fig.14.10 show the adaptation of the number of somites to different sizes of the field and that the somite formation can proceed normal in a posterior fragment.

14.8 The problem of segmentation

As an open problem remains the question what is the signal to form a border between two segments or the cleft between two somites? Our assumption was that each of these repetitive substructures consists of an A and a P part. A

segment border coincides with a P-A border. However, a juxtaposition of A and P cells cannot be the signal to form a segment border (or a cleft), since a second A-P confrontation is present in the center of each segment, without a segment border being induced. Even if a pair of A-P stripes always has to form a segment, the grouping of a sequence APAPAP would be ambiguous; either AP/AP/AP... or A/PA/PA/P... would be possible. The internal polarity within the segment would not be determined. In insects segment border coincides with a P-A border. However, a juxtaposition of A and P cells cannot be the signal to form a segment border (or a cleft) since a second A-P confrontation is present in the center of each segment, without a segment border being induced. Even if a pair of A-P stripes always has to form a segment, the grouping of a sequence APAPAP would be ambiguous either AP/AP/AP... or A/PA/PA/P... would be possible. The internal polarity within the segment would not be determined.

The Bithorax gene complex has provided us with very important insights how segmentation is *not* controlled. As mentioned, several mutations (Fig.14.3) cause the specification of a particular segment to extend into a neighboring segment. The segment border no longer coincides with the transition from one segmental specification to the next. In other words, a transition from one segmental specification to the next, for instance from mesothoracic to metathoracic specification, can occur within a segment without a segment border being induced. We have to conclude that a segment border is not induced by a transition from one segmental specification to the next. After a complete deletion of the Bithorax complex, the metathoracic and all abdominal segments have mesothoracic specificity. However, the total number of segments remain unchanged. Thus, segmentation proceeds independently of whether the segments are different from each other or not. An assumption that more and more segments are added until the last abdominal segment is present is obviously incompatible with this observation. Counting of segments and giving them individual specifications are two different processes.

If neither the transition from one segmental specification to the next nor the P-A confrontation is the signal to form a segment boundary the question remains: what is the signal? One solution of this problem could be that the primary building blocks of a segment or a somite are not two states, A and P but three, for instance A, P and S (segment border). The primary periodic pattern formation would lead to an ...APSAPS... sequence which allows a segmentation either of the type .../APS/APS/... or .../SAP/SAP/..., depending whether S/A or P/S induces a border. The advantage of having a subdivision into three parts is twofold. On one hand, the determination of the segment border is unequivocal, for instance SAP/SAP/... Secondly, the internal polarity of the segment is well-defined. No other grouping is possible. The sequences /SAP/ and /PAS/ have opposite polarities. We have seen in chapter 12 and 13 how several structures in a sequence can be stabilized. The basic principle was that different states, for instance S, A and P, exclude each other locally but stabilize each other on long range. For instance, on long range S supports A, A supports P and P supports S and/or vice versa. This leads to a repetitive SAPSAP... pattern.

A direct evidence for three such building blocks of a segment is not yet avail-

able but several experimental observations would find a straightforward explanation under this assumption. Nüsslein-Volhard and Wieschaus (1980) found a mutation in *Drosophila* in which twice as many segment borders are formed and in which the internal organization of the segments appears to be symmetric. Such a phenotype is expected if the central state is affected by the mutation. For instance, a /SAP/ pattern would lead, if the state A is affected, to a pattern /S/P/S/P/. Further, if an anterior and a posterior part of an abdominal segment of a bug is juxtaposed, a new segment boundary is formed (Wright and Lawrence, 1981a,b). The same happens after removal of a large internal part of a leg segment (French, 1976a). In the model, if the A area is removed from an /SAP/ sequence, the newly formed SP confrontation would induce a new boundary. The fact that a threefold subdivision has not yet been found is not an argument against this possibility. The A-P subdivision in the thoracic segments has been discovered due to the clonal restriction. However, the sharp AP boundary in the thoracic segments may be more the exception than the rule and required solely to define the coordinate system for appendages (chapter 9). No clonal restriction is necessarily present between the other states. To the contrary, some diffusion facilitates the size regulation of the individual elements (see Fig.12.6) and in the abdominal segments, no compartments have been found (Lawrence et al., 1978).

An open problem is further how the precise number of segments is controlled. The formation of the segments must be under the control of the primary gradient since the shallower gradient in double abdomen embryos (Fig.8.5, 8.3) or in ligated eggs (Fig.8.7) leads to fewer segments. A key observation are mutants in which each second segment is skipped, either the even numbered or the odd numbered (Nüsslein-Volhard and Wieschaus, 1980; Sander et al., 1980). A coherent model for this phenomenon is still missing.

14.9 Stepwise modification under the influence of A-P-A alternations

The activation of a next control gene under the influence of a P-A transition is only one of several possibilities. The Bithorax complex indicates that this possibility is realized in the insect system. We do not know how general this mechanism of activating particular control genes really is. Another possibility would be a systematic modification, for instance by a somatic processing of particular DNA or RNA sequences. The alternation between P-A-P... could control the occurrence and the spatial distance of the modifications. The signal "modify" may require a simultaneous high A and P concentration. Only during the very short period of transition between A and P are both states active within the same cell since the states A and P mutually exclude each other. If an A-specific and a P-specific enzyme have to be present simultaneously to accomplish a particular biochemical step, this can take place only in the short phase of transition. A homeostatic maintenance of the once attained state would result since, after completion of the pattern, the cell would remain stably in one of the states and

transitions would no longer occur.

14.10 The advantage of having a superposition of periodic and sequential structures

The model provides an explanation about how periodic and sequential structure can be formed. The gating and counting mechanism provides a mechanism to produce a large number of similar but different structures. Due to the superposition of the two patterns, the precision by which the morphogen concentration has to be measured is much reduced since the fine structure and correct neighborhood emerges under the control of the periodic pattern with its higher spatial resolution. The compartments formed in this process are not only involved in gating the control genes. By cooperation of compartments, the A-P pattern can determine the position and orientation of appendages (chapter 9). Alternatively the A- and P-stripes may act as the terminal structures within abdominal segments and the missing structures are filled in by intercalation (chapter 13). In any case, the superposition of both the periodic and sequential structure provides preconditions for making a reliable finer subdivision of a developing organism.